Data Evaluation Report on the Acute Toxicity of Ethylene Urea (Degradate of ETU) to Freshwater Invertebrates - *Daphnia magna*

PMRA Submission Number{.....} EPA MRID Number 464629-03

Data Requirement:

PMRA DATA CODE	{.....}
EPA DP Barcode	D305127
OECD Data Point	
EPA MRID	464629-03
EPA Guideline	850.1010

Test material: Ethylene Urea, a degradate of Ethylene Thiourea (ETU) **Purity:** 96.0%
Common name: EU
Chemical name: IUPAC: Not reported
CAS name: Not reported
CAS No.: Not reported
Synonyms: Not reported

Primary Reviewer: John Marton
Staff Scientist, Dynamac Corporation
Signature:
Date: 3/14/2005

QC Reviewer: Gregory Hess
Staff Scientist, Dynamac Corporation
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Date: 4/18/2005

Primary Reviewer: Brian Montague
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Date: February 23 2015

Secondary Reviewer(s):
{EPA/OECD/PMRA} Allen W. Vaughan
Date: 04/16/15

Reference/Submission No.:

Company Code:
Active Code:
EPA PC Code: No PC code for degradate (PC code of ETU: 600016)

Date Evaluation Completed: 02-13-2015

CITATION: Palmer, S.J., Kendall, T.Z. and Krueger, H.O. 2001. Ethylene Urea: A 48-Hour Static Acute Toxicity Test With The Cladoceran (*Daphnia magna*). Unpublished study performed by Wildlife International, Ltd., Easton, Maryland. Laboratory Project No. 299A-114. Study submitted by EBDC/ETU Task Force, Washington, D.C.. Study completed January 26, 2001.

EXECUTIVE SUMMARY:

The 48-hour acute toxicity of Ethylene Urea (EU) to the water flea, *Daphnia magna*, was studied under static conditions. Daphnids were exposed to the test material at nominal concentrations of 0 (negative control), 130, 216, 360, 600 and 1000 ppm a.i.. Mean-measured treatment concentrations were <5.00 (<LOQ; negative control), 126, 214, 354, 589 and 985 ppm a.i., and were 97.1-99.2% of nominal. The nominal treatment concentrations were adjusted to 100% based on the reported purity of 90.8%.

After 48-hours of exposure, mortality was 0% in the negative control and mean-measured 126 through 589 ppm a.i. treatment groups, and 10% in the mean-measured 985 ppm a.i. treatment group. All surviving daphnids appeared normal after 48-hours. The 48-hour LC_{50}/EC_{50} was >985 ppm a.i., which categorizes Ethylene Urea as practically non-toxic to the water flea (*Daphnia magna*) on an acute toxicity basis. The 48-hour NOEC and LOEC levels were 589 and 985 ppm a.i., respectively, based on the mean-measured concentrations.

This study is scientifically sound and fulfills U.S. EPA guideline §72-2a for an acute toxicity study with freshwater invertebrates. This study is classified as ACCEPTABLE.

Results Synopsis

Test Organism Age (eg. 1st instar): <24 hours old
Test Type (Flow-through, Static, Static Renewal): Static

48-Hour
LC_{50}/EC_{50}: >985 ppm a.i. 95% C.I.: N/A
Slope: N/A
NOEC: 589 ppm a.i.
LOEC: 985 ppm a.i.
Endpoints affected: Mortality

I. MATERIALS AND METHODS

GUIDELINE FOLLOWED: The study protocol was based on procedures outlined in OECD Guideline for Testing of Chemicals, 202: *Daphnia sp. Acute Immobilization and Reproduction Test* and ASTM Standard E729-88a, *Standard Guide for Conducting Acute Toxicity Tests with Fishes, Macroinvertebrates and Amphibians*. Deviation from §72-2a included:

1. The reported dilution water hardness (136 mg/L as $CaCO_3$) was higher than recommended (40-48 mg/L as $CaCO_3$) at test initiation. The pH range (8.2-8.6) was higher than recommended (7.2-7.6).

2. The TOC, particulate matter, and chlorine concentrations within the dilution water were not reported.

3. The biomass loading rate was not specified.

4. It was not reported whether or not the test vessels were aerated during the definitive test.

The above deviations did not affect control survival or the acceptability or the validity of this study.

COMPLIANCE: A signed and dated GLP, No Data Confidentiality, and Quality Assurance statements were provided. This study was conducted in accordance with GLP standards of the U.S. EPA (40 CFR Part 160-FIFRA), OECD ENV/MC/CHEM(98)17, and Japan MAFF, 59 NohSan, Notification No. 3850, Agricultural Production Bureau, 10 August 1984.

A. MATERIALS:

1. Test Material Ethylene Urea (Degradate of Ethylene Thiourea)

Description: Off-White Crystalline Solid

Lot No./Batch No. : 01743-141

Purity: 90.8%

Stability of Compound Under Test Conditions: The stability of the test substance in dilution water was demonstrated by analytical determination on Day 0 (97.1-99.4% of nominal) and Day 2 (96.3-100% of nominal), which resulted in mean-measured recoveries of 97.1-99.2% of nominal. The study authors reported that the nominal concentrations were adjusted to 100% based on the reported purity of 90.8%.

OECD requires water solubility, stability in water and light, pK_a, P_{ow}, and vapor pressure of the test compound. The OECD requirements were not reported..

Storage conditions of test chemicals: Stored under ambient conditions.

2. Test organism:
Species: *Daphnia magna*
Age at test initiation: Neonates, <24 hours old
Source: In-house laboratory cultures.

B. STUDY DESIGN:

1. Experimental Conditions

a) Range-finding Study: Nominal concentrations used in the definitive test were selected in consultation with the Sponsor, and were based upon the results of exploratory range-finding toxicity tests; results from these studies were not provided.

b) Definitive Study:

Table 1 . Experimental Parameters

Parameter	Details	Remarks *Criteria*
Acclimation period: Conditions: (same as test or not) Feeding: Health: (any mortality observed)	Continuous laboratory cultures were maintained. Same as test *Daphnia* cultures were fed a mixture of yeast, Cerophyll® and trout chow, as well as a suspension of the alga *Selenastrum capricornutum* daily. Adults showed no signs of disease or stress.	Daphnids were acclimated for 14 days preceding the test (Temp: 19.9-21.4°C, pH: 8.3-8.5; and DO: 8.3-8.9 mg/L). The adult daphnids were fed prior to the test, but neonates were not fed during testing. *EPA requires 7 day minimum acclimation period. No feeding during study.*
Duration of the test	48 hours	*EPA requires 48 hours*
Test condition - static/flow through Type of dilution system (for flow through method) Renewal rate (for static renewal)	Static N/A N/A	*EPA requires consistent flow rate of 5 - 10 volumes/24 hours, meter systems calibrated before study and checked twice daily during test period*
Aeration, if any	None reported.	
<u>Test vessel</u> Material: *(glass/stainless steel)* Size: Fill volume:	Glass beakers 250 mL 250 mL	*EPA requires: size 250 ml or 3.9 L fill 200 ml*
Source of dilution water	Moderately-hard water was obtained from a 40 m deep on-site well. Water was sand-filtered to remove particles greater than approximately 25µm, aerated, then filtered again to remove microorganims and particles (0.45 µm).	*EPA requires soft reconstituted water or water from a natural source, not dechlorinated tap water.*
<u>Water parameters</u>:		The hardness (136 mg/L as $CaCO_3$)

Parameter	Details	Remarks *Criteria*
Hardness pH Dissolved oxygen Temperature Total Organic Carbon Particulate matter Metals Pesticides Chlorine	136 mg/L as $CaCO_3$ 8.2-8.6 (7.9-8.1 in 4 weeks prior to test) 8.5-9.2 mg/L (≥94% saturation) 20.1-20.8°C Not reported Not reported <LOD <LOD Not reported	was higher than recommended (40-48 mg/L as $CaCO_3$). The well water pH (8.2-8.6) ranged higher than recommended (7.2-7.6). Alkalinity and Conductivity were 182 mg $CaCO_3$/L and 300 µmhos/cm, respectively. Magnesium, sodium, calcium and potassium were detected in the dilution water at concentrations of 11.0, 18.0, 29 and 1.1 mg/L, respectively. *EPA requires:* *hardness: 40 - 48 mg/L as $CaCO_3$* *pH: 7.2 - 7.6* *-Temperature: 20°C (measured continuously or if water baths are used, every 6 hr, may not vary > 1°C* *Dissolved oxygen:* *Static: ≥60% during 1st 48 hr and ≥40% during 2nd 48 hr* *Flow-through: ≥60%*
Number of replicates Solvent control: Negative control: Treatments:	 N/A 4 4	
Number of organisms per replicate Solvent control: Negative control: Treatments:	 N/A 5/replicate, 4reps./level 5/replicate, 4reps./level	The biomass loading rate was not specified. *EPA requires 5 treatment levels plus control with a minimum of 20 daphnid per treatment. Biomass loading rate for static ≤0.8 g/L at ≤17°C, ≤0.5 g/L at > 17°C; flow-through: ≤1 g/L/day.*
Treatment concentrations nominal: measured:	 0 (negative control), 130, 216, 360, 600 and 1000 ppm a.i. <5.00 (<LOQ; negative control), 126, 214, 354, 589 and 985 ppm a.i.	Samples were collected from two alternating replicate test chambers of each treatment and control group at 0 and 48 hours. The study authors reported that the nominal concentrations were adjusted to 100% based on the reported purity of 90.8%.

Parameter	Details	Remarks *Criteria*
		EPA requires a geometric series with each concentration being at least 60% of the next higher one.
Solvent (type, percentage, if used)	N/A	*EPA requires solvents not to exceed 0.5 mL/L for static tests or 0.1 mL/L for flow-though tests.*
Lighting	16-hours light/8-hours dark with a 30 min transition period of low light intensity.	Light intensity at test initiation was approximately 304 lux at the surface of one representative test chamber.
		EPA requires 16 hours light, 8 hours dark.
Stability of chemical in the test system	Stable, based on mean analytical recoveries from 0 and 48 hours.	Recoveries were 97.1-99.4% of nominal concentrations at 0 hours and 96.3-100% at 48 hours.
Recovery of chemical Level of Quantitation Level of Detection	97.1-99.9% of nominal 5.00 ppm a.i. Not reported	Based on QC matrix fortifications of 0 (blank matrix), 100, 250 and 1000 ppm a.i.
Positive control {if used, indicate the chemical and concentrations}	N/A	
Other parameters, if any	N/A	

2. Observations:

Table 2: Observations

Criteria	Details	Remarks *Criteria*
Parameters measured including the sublethal effects	Immobility/mortality and sub-lethal effects	
Observation intervals	After 20, 24 and 48 hours	
Were raw data included?	Yes, sufficient	
Other observations, if any	N/A	

II. RESULTS AND DISCUSSION

A. MORTALITY:

After 48-hours of exposure, mortality was 0% in the negative control and mean-measured 126 through 589 ppm a.i. treatment groups, and 10% in the mean-measured 985 ppm a.i. treatment group. The reported 48-hour LC_{50}/EC_{50} (with 95% C.I.) was >985 (N/A) ppm a.i. and the NOEC for mortality/immobility was 589 ppm a.i. based on the mean-measured treatment concentrations.

Table 3: Effects of Ethylene Urea on Mortality/Immobilization of *Daphnia magna.*

Treatment, ppm a.i. 48-Hour Mean-Measured and (Nominal) Concn.	Observation Period					
	20 Hours		24 Hours		48 Hours	
	No. Dead	% Affected	No. Dead	% Affected	No. Dead	% Affected
Negative Control	0	0	0	0	0	0
126 (130)	0	0	0	0	0	0
214 (216)	0	0	0	0	0	0
354 (360)	0	0	0	0	0	0
589 (600)	0	0	0	0	0	0
985 (1000)	0	0	0	0	2	10
NOEC, ppm a.i.	589 [1]					
LOEC, ppm a.i.	985 [1]					
EC_{50} (with 95% C.I.), ppm a.i.	>985 (N/A)[1]					

[1] The reported toxicity values were determined in terms of the mean-measured treatment concentrations.

B. SUB-LETHAL TOXICITY ENDPOINTS:

After 48-hours of exposure, all surviving daphnids were reported to be normal in the negative control and all treatment groups. The reported 48-hour EC_{50} (with 95% C.I.) was >985 (N/A) ppm a.i. and the NOEC for sub-lethal effects was 985 ppm a.i. based on the mean-measured treatment concentrations.

Table 4. Sub-Lethal Effects of Ethylene Urea on Water flea (*Daphnia magna*).

Treatment, ppm a.i., Mean-Measured and (Nominal) Concentration	Observation Period		
	Endpoint at 20 Hours	Endpoint at 24 Hours	Endpoint at 48 Hours
	% Affected[1]	% Affected	% Affected
Negative control	A.N.	A.N.	A.N.
126 (130)	A.N.	A.N.	A.N.
214 (216)	A.N.	A.N.	A.N.
354 (360)	A.N.	A.N.	A.N.
589 (600)	A.N.	A.N.	A.N.
985 (1000)	A.N.	A.N.	A.N.
NOEC (sub-lethal)	985 ppm a.i.[2]		
LOEC (sub-lethal)	>985 ppm a.i.[2]		
EC_{50}	Not reported		
Positive control, if used % sub-lethal effect: EC_{50}:	N/A	N/A	N/A

[1] % Affected is the number of daphnids exhibiting symptoms/number of surviving daphnids x 100.
N/A = Not Applicable; A.N.=All Appear Normal
— = 100% Mortality
[2] The reported toxicity values were determined in terms of the mean-measured treatment concentrations.

C. REPORTED STATISTICS:

Statistical Method: The concentration-response pattern observed in the study precluded the statistical calculation of an LC_{50}/EC_{50} value. Therefore the LC_{50}/EC_{50} and NOEC values were determined by visual interpretation of the mortality, immobility and observation data. All toxicity values were determined in terms of the mean-measured treatment concentrations.

48-Hour
LC_{50}/EC_{50}: >985 ppm a.i. 95% C.I.: N/A
Slope: N/A
NOEC: 589 ppm a.i.
LOEC: 985 ppm a.i.
Endpoints affected: Mortality

D. VERIFICATION OF STATISTICAL RESULTS:

Statistical Method: The 48-hour LC_{50}/EC_{50}, NOEC and LOEC values were determined visually due to lack of mortality and sub-lethal effects in the negative control and the mean-measured 126 through 589 ppm a.i. treatment groups and the observed 10% mortality at the 985 ppm a.i. treatment level by 48-hours. All toxicity values were determined in terms of the mean-measured treatment concentrations.

48-Hour
LC_{50}/EC_{50}: >985 ppm a.i. 95% C.I.: N/A
Slope: N/A
NOEC: 589 ppm a.i.
LOEC: 985 ppm a.i.
Endpoints affected: Mortality

E. STUDY DEFICIENCIES:

All deviations from U.S. EPA guideline §72-2a were considered minor and did not affect validity or acceptability this study.

F. REVIEWER'S COMMENTS:

The results of the reviewer's statistical verification were identical to those of the study author.

The nominal test concentrations were adjusted to 100% based on the reported test substance purity (90.8%).

The DO concentration and pH of the dilution water were measured in replicate A at 20-hours, in replicate B at 24 hours and in replicate C at 48-hours.

G. CONCLUSIONS:

This study is scientifically sound, fulfills U.S. EPA guideline 850.1010 (previously FIFRA 72-2) and is classified as ACCEPTABLE. Based on the results of this study (LC_{50}/EC_{50} of >985 ppm a.i.), Ethylene Urea is categorized as practically non-toxic to the water flea, *Daphnia magna*, on an acute toxicity basis.

48-Hour
LC_{50}/EC_{50}: >985 ppm a.i. 95% C.I.: N/A
Slope: N/A
NOEC: 589 ppm a.i.
LOEC: 985 ppm a.i.
Endpoints affected: Mortality

III. REFERENCES:

Federal Register: 43 FR 29696, Subpart E- Hazard Evaluation: Wildlife and Aquatic Organisms, 163. 72-1 Fish Acute LC_{50} , July 10, 1978.

ASTM Publication E729-80. "Standard Practice for Conducting Acute Toxicity Tests with Fishes, Macroinvertebrates and Amphibians." American Society for Testing and Materials, 1916 Race Street, Philadelphia PA 19103, May 1980.

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